

any cells showed the same signal intensity (data not shown). After an incubation time of 16 hours at 37 degrees C., the emulsions were re-injected into the chip together with additional fluorinated oil (separately injected into the oil inlet to space out the drops) to repeat the fluorescence measurement (at  $t_2$ ; analyzing 500 drops per second). Plotting the maximum fluorescence intensity of the drops against the peak width (which corresponds to the drop size and therefore is an indicator of coalescence) revealed different distinct populations (FIG. 15F). Analysis of the peak width proved that even though populations with two-fold and three-fold higher volumes were observable, the majority of drops did not coalesce (>93%). In terms of the fluorescence two main populations were obtained having a roughly 35-fold difference in their intensity, as also confirmed by fluorescence microscopy in which the drops appeared to be either highly fluorescent or non fluorescent (FIG. 15C). Based on these observations gates were set for the quantitative interpretation of the data (as routinely done in FACS analysis). Gates were set to analyze only the drops which had not coalesced (corresponding to the populations with the lowest peak width). Based on the way the peak width was defined fluorescence-positive drops appeared to be bigger (see FIG. 15E). Nonetheless, plotting the fluorescence against the peak width enabled non-coalesced drops to be clearly distinguished from coalesced drops for both species (positives and negatives). Using gating led to the conclusion that roughly 5.08% of all non-coalesced drops were fluorescence positive in the sample with non-diluted transduced cells. This number corresponded to approximately 12.7% of the corresponding cell population when taking into account that only 40.0% of the drops were occupied (as determined by microscopical analysis of the drops during the encapsulation step). This value was in the same range as the fraction of positive cells determined in bulk (~13.9%), using a conventional X-Gal assay. For the diluted sample 0.63% positive drops were obtained, corresponding to 1.8% of the cells (34.8% of the drops were occupied). Compared to the non-diluted sample, the negative population showed a lower fluorescence intensity. Not wishing to be bound by any theory, this may have been due to the fact that all drops (even the ones without cells) contained traces of soluble  $\beta$ -galactosidase resulting from the few dead cells within the syringe (during the encapsulation step). Since the diluted sample contained less enzyme in total, a lower background could be expected, too. Another possible explanation would be the exchange of fluorescein between the drops. However, this explanation seems to be less likely, since for incubation periods of up to 24 hours, significant exchange of fluorescein were not observed for all surfactants tested (including the ammonium salt of carboxy-PFPE and PEG-PFPE; data not shown). The resulting 7.1-fold difference in terms of positive cells between the samples was in good agreement with the initial 1:9 dilution (assuming an accuracy of  $\pm 10\%$  when counting the cultures in a Neubauer chamber before mixing leads to the conclusion that the effective ratio might have been as low as 1:7.4). In summary, these results clearly demonstrated the possibility of quantitatively analyzing individual drops in a high-throughput fashion (the drops were analyzed at a frequency of 500 Hz).

**[0231]** Droplet-based microfluidic systems have been used to create miniaturized reaction vessels in which both adherent and non-adherent cells can survive for several days. Even though microcompartments were generated with volumes of 660 pl and 660 nl only, in principal almost any volume could

be generated by changing the channel sizes and flow rates, or by splitting relatively large microcompartments through a T-junction into smaller units. Thus microcompartments tailored for the encapsulation of small objects like single cells could be generated as well as compartments big enough to host multicellular organisms like *C. elegans*. Furthermore, the size could be adjusted according to the assay duration. Cell density was found to inversely correlate with the survival time of encapsulated cells. Larger compartments are hence preferential for long-term assays, especially since encapsulated cells proliferate within the microcompartments. Consequently even proliferation assays (e.g. for screening cytostatic drugs) should be possible as long as the chosen volume is big enough to guarantee sufficient supply of nutrition. On the other hand, small volumes might be advantageous for other applications, for example, to minimize reagent costs or to rapidly obtain high concentrations of secreted cellular factors. Besides the volume, further factors have been shown to have an impact on cell-survival, notably the biocompatibility of the surfactants and the gas-permeability of the storage system. Both non-ionic surfactants described herein allowed cell survival and proliferation, whereas the two ionic surfactants mediated cell-lysis. Even though there is no direct proof of correlation, it was striking that poly-L-lysine, a compound widely used to improve cell-attachment to surfaces, mediated membrane disruption when used as a head group of an ionic surfactant. Long-term incubation also requires sufficient gas-exchange. This can be ensured either by using open reservoirs, or channels or tubing made of gas-permeable materials such as fluorinated polymers. Efficient gas-exchange is also helped by the fact that perfluorocarbon carrier fluids can dissolve more than 20 times the amount of  $O_2$ , and three times the amount of  $CO_2$ , than water and have been shown to facilitate respiratory gas-delivery to both prokaryotic and eukaryotic cells in culture.

**[0232]** The possibility of re-injecting microcompartments into a chip after the incubation step opens the way for integrated droplet-based microfluidic systems for cell-based high-throughput screening. As has been shown here, a fluorescence-based readout of the expression of a cellular reporter gene can be performed in individual compartments at frequencies of 500 Hz. Hence a wide range of commercially-available fluorescence-based assays, can potentially be performed in a high throughput fashion. It is noteworthy that the possible coalescence of individual drops does not necessarily bias the readout. As shown here, coalesced drops with higher volumes can easily be identified and excluded from the data analysis. In theory, the use of gates also allows the analysis of solely those compartments hosting a specific number of (fluorescent) cells. In contrast to conventional FACS analysis the assay readout does not have to be based on fluorophores which remain in, or on the surface of the cells (e.g. GFP or fluorescent antibodies). Using compartmentalization, the activity of an intracellular reporter enzyme ( $\beta$ -galactosidase) has been measured using a fluorescent product that is highly membrane permeable (fluorescein).

**[0233]** The integration of additional microfluidic modules to the microfluidic platforms shown here allows the application range to be expanded. Integrating a microfluidic sorting module (based on dielectrophoresis or valves) could, for example, enable the screening of drug candidates. In the simplest case the candidates could be genetically-encoded by the encapsulated cells themselves (starting with a cell library): hence the collection of sorted positive drops would